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AWARD NUMBER DAMD17-97-1-7120

TITLE: New Rhenium Radiopharmaceuticals for the Selective Imaging
and Therapy of Breast Cancer

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REPORT DATE: June 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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DTIC QUALITY INSPECTED 1

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 1998	3. REPORT TYPE AND DATES COVERED Annual (12 May 97 - 11 May 98)	
4. TITLE AND SUBTITLE New Rhenium Radiopharmaceuticals for the Selective Imaging and Therapy of Breast Cancer			5. FUNDING NUMBERS DAMD17-97-1-7120	
6. AUTHOR(S) Arterburn, Jeffrey B., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) New Mexico State University Las Cruces, New Mexico 88003-8001			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) <p>This report describes progress achieved during year-1 of a three year project, which seeks to develop new technology to produce rhenium radiopharmaceuticals for the diagnostic imaging and therapy of breast cancer. The discovery of a new palladium-catalyzed synthetic routes to alkynyl- and alkenyl-linked estradiol hydrazine derivatives has been a major advance. This reaction forms a new carbon-carbon bond between ethynyl- or vinyl estradiol derivatives and N'-(acyl)-4-iodophenylhydrazine, incorporating the hydrazine group in excellent yields without side reactions or degradation of the steroid. The reaction was used to prepare analogs attached to a polymeric support. The desired reactivity of these hydrazine compounds with the rhenium complex $\text{ReOCl}_3(\text{PPh}_3)_2$ has also been shown, leading to the first generation of target organoimidorhenium-estradiol complexes. These studies provide proof of concept for the fundamental hypothesis of this project which is that solid-supported imido precursors can be used to synthesize rhenium radiopharmaceuticals without requiring additional steps to remove unlabeled ligand.</p>				
14. SUBJECT TERMS Breast Cancer RADIOPHARMACEUTICAL, THERAPY, IMAGING, ESTRADIOL RHENIUM			15. NUMBER OF PAGES 12	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

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5. INTRODUCTION:

This report describes work completed on award number DAMD17-97-1-7120 during the first year of the project (May 12, 1997- May 11, 1998). This project involves a fundamental investigation of new compounds and the development of new synthetic methodology for the imaging and radiotherapy of breast cancer with rhenium-188 labeled estradiol derivatives. Research efforts during the first year have focused on Technical Objective #1, as described in the original proposal text, and reproduced immediately below this paragraph. We have attained our initial goals of producing estradiol precursors and attaching these to polymeric supports. We have also extended our efforts to include successful Re-labeling to produce the first generation of target organoimidorhenium-estradiol complexes.

Technical Objective #1: Synthesize a series of polymer supported estradiol ligand precursors.

Task 1: Months 1-6.

A variety of estradiol precursors will be synthesized in preparation for attachment to the polymer supports. Representative compounds possessing azide/phosphinimine, acylhydrazine, and alkoxyamine groups will be prepared and fully characterized.

Task 2: Months 7-12

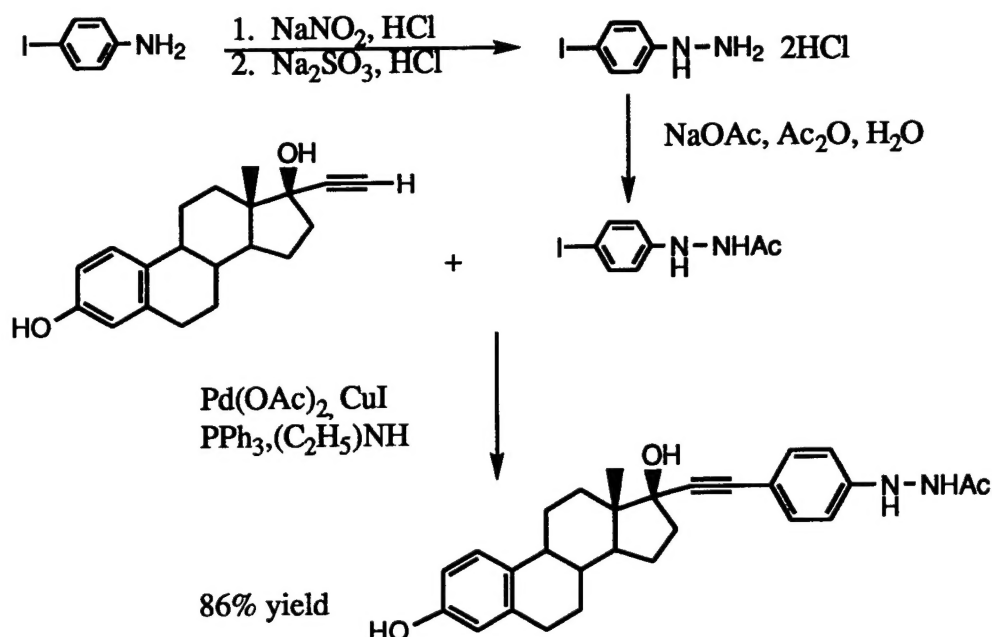
The estradiol imido precursors will be attached to commercially available functionalized polymers. Several strategies and reaction conditions for incorporating the imido precursors into the polymers will be investigated to identify the best methods of preparation. Rhenium complexes to be in the next phase of the study used will be prepared.

6. BODY:

Methods. All experiments were performed in a fume hood. Air and moisture-sensitive reactions were performed on a vacuum line under an argon atmosphere using freshly dried and distilled solvents. All other reactions were carried out using solvents and reagents without any further purification. All solvents were purchased from Aldrich, Acros, or Baker. Reagents were purchased from Aldrich or Acros, except KReO_4 which was purchased from Johnson-Matthey. Deuterated solvents were purchased from Aldrich or Cambridge Isotope Laboratories and were used without and further purification except for d_6 -DMSO which was dried over sieves. The compound $\text{Re}(\text{O})\text{Cl}_3(\text{PPh}_3)_2$ was prepared according to published procedure.¹

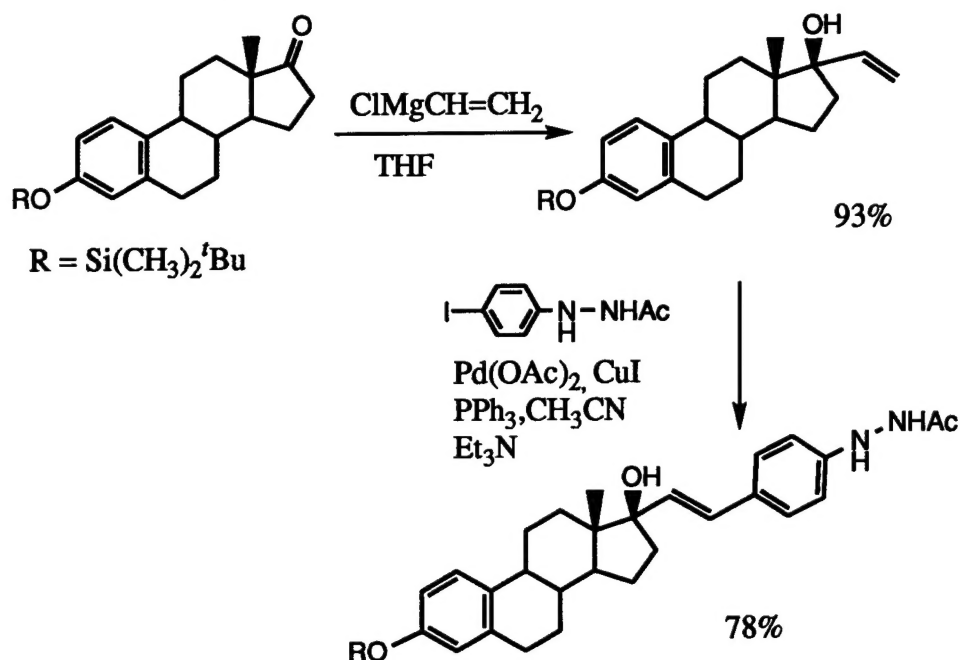
NMR spectra were acquired at ambient temperatures (24 ± 2 °C) unless otherwise noted using Varian Unity 400 and Gemini 200 Fourier transform spectrometers. The ^1H NMR were referenced to TMS or the residual protons of the solvent. The $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were recorded at 161.9 and referenced relative to an external standard of 85% H_3PO_4 . The $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were recorded at 50 MHz or 100 MHz and referenced relative to the $^{13}\text{C}\{^1\text{H}\}$ peaks of the solvent. Spectra are reported as δ (ppm) (number of hydrogens, multiplicity assignment). Infrared spectra were recorded on a Perkin-Elmer 1720X FT-IR as KBr pellets and are reported in cm^{-1} .

Results/Discussion. The primary technical goal addressed during project year-1 was the synthesis of estradiol derivatives possessing nitrogenous imido precursors. Our previous experience using acetylhydrazines as imido precursors for preparing ^{188}Re organoimido complexes led us to focus our synthetic design exclusively on this class of compounds.² Commercially available 17α -ethynylestradiol was selected as the steroid starting material. Our desire for a concise, efficient synthesis of the target compounds led us to develop the new palladium-catalyzed reaction between 17α -ethynylestradiol and N -(acetyl)- N' -4-iodophenylhydrazine shown in Scheme 1.

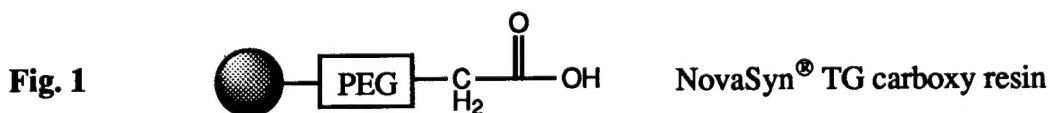
Scheme 1. Synthesis of Ethynyl-Estradiol-Hydrazine Imido Precursors

This synthesis is remarkably concise, forming a new bond between the terminal alkyne carbon and the aryl carbon of the phenylhydrazine derivative, while the acetylhiazine unit was incorporated intact, without side reactions or degradation of the sensitive phenol and tertiary alcohol functional groups of the steroid. This approach avoids the requirement for additional bifunctional crosslinking agents, which are typically used to prepare bioconjugates. Similar reactions are observed when the phenolic *t*-butyl-dimethylsilyl ether are used, and also with the *t*-butoxycarbonyl- (BOC)-derivative of 4-iodophenylhydrazine. The carbon-carbon triple bond in these alkynyl-linked hydrazines provides a rigid, linear rod-like connection between the estradiol unit and the metal bonding site.

In order to have access to "bent" ethenyl-linked hydrazines we developed a palladium catalyzed reaction involving vinylestradiol as shown in Scheme 2. The *t*-butyl-dimethylsilyl protected vinylestradiol was prepared by addition of vinylmagnesium chloride to the protected estrone derivative. The palladium-catalyzed Heck reaction with *N*-(acetyl)-*N'*-4-iodophenylhydrazine gave the alkenyl-linked hydrazine product.

Scheme 2. Synthesis of Ethenyl-Estradiol-Hydrazine Imido Precursors

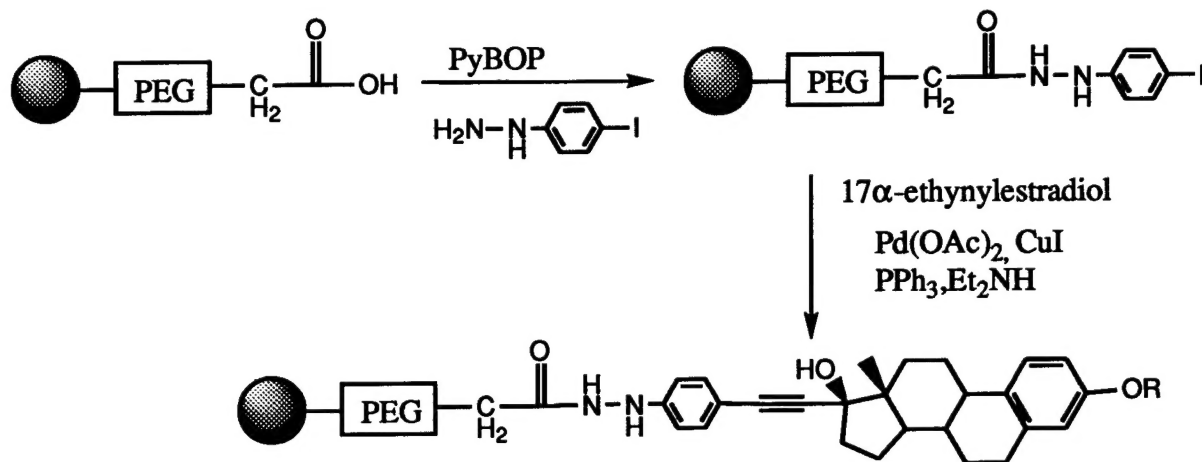
These two palladium-catalyzed hydrazine syntheses were also ideally suited for our goal of attachment to polymeric supports. We selected NovaSyn's tentagel carboxy resin (TG carboxy resin) as the polymeric support for this investigation because of its ability to perform in a wide variety of solvents, ranging from water to toluene. This resin consists of a polystyrene backbone conjugated to polyethyleneglycol chains which are terminated with carboxylic acid groups (Fig. 1).



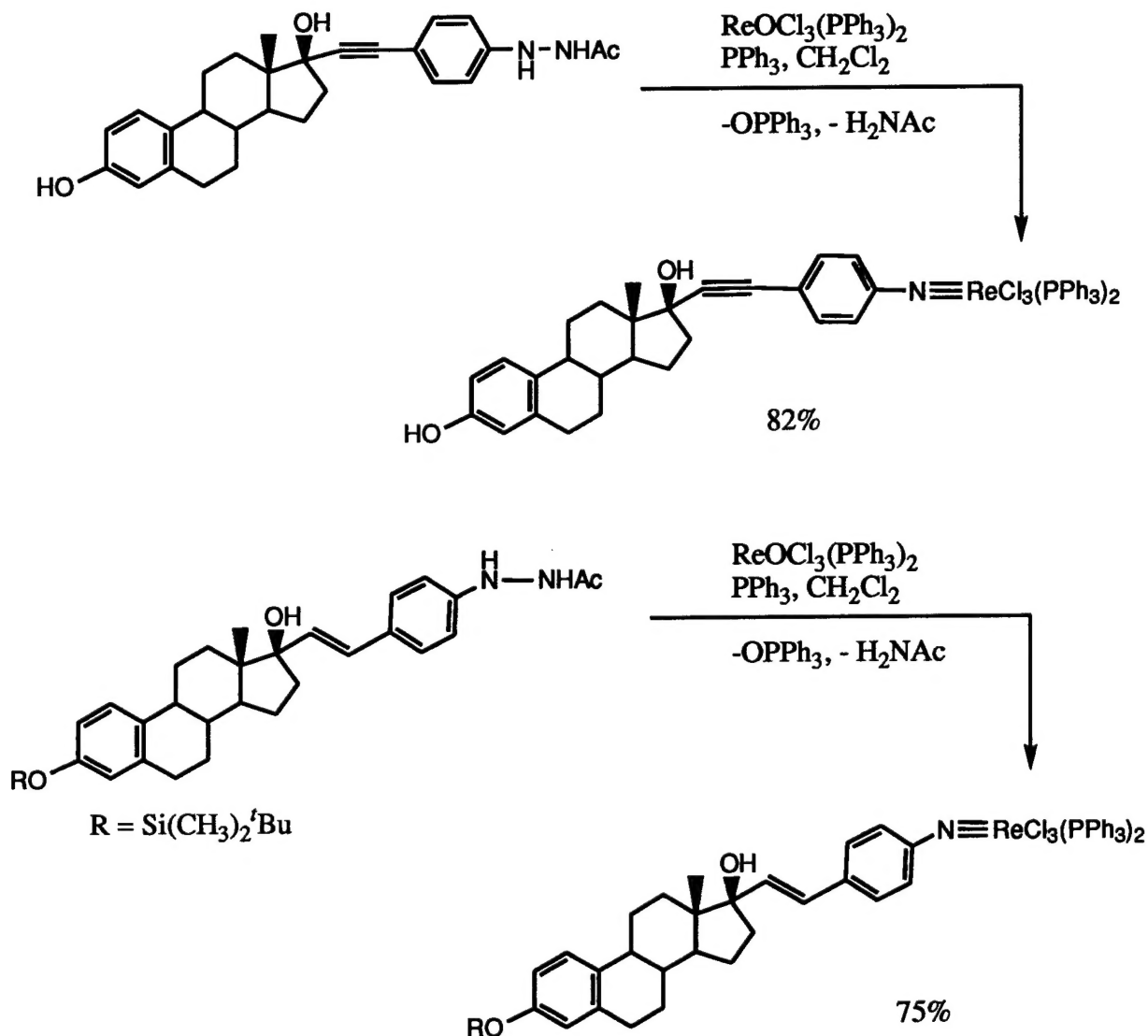
Our synthesis of the solid supported alkynyl-linked hydrazine estradiol derivative is shown in Scheme 3. The TG carboxy resin was coupled with 4-iodophenylhydrazine under the influence of benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP®). The reaction was monitored by FT-IR spectroscopy following the change in the carbonyl stretch from the free acid (1737 cm^{-1}) to the acylhydrazine (1660 cm^{-1}). The palladium-catalyzed alkyne coupling with 17α -ethynylestradiol was carried out under the same conditions as the reactions performed in solution. The successful carbon-carbon coupling was evident by the appearance of a new FT-IR absorbance at 1611 cm^{-1} attributed to

the aryl A-ring of the steroid, and also observed in the spectra of the acylhydrazine derivative prepared previously in solution.

Scheme 3. Synthesis of Solid-Supported Ethenyl-Estradiol-Hydrazine Imido Precursors



With the first generation of estradiol derivatives made available by the syntheses described above, we initiated rhenium labeling studies with stable isotope $\text{ReOCl}_3(\text{PPh}_3)_2$ in order to prepare the target organoimido complexes. Optimized reaction conditions were rapidly developed using the soluble ethynyl- and ethenyl-linked acylhydrazine derivatives in dichloromethane solvent as shown in Scheme 4.

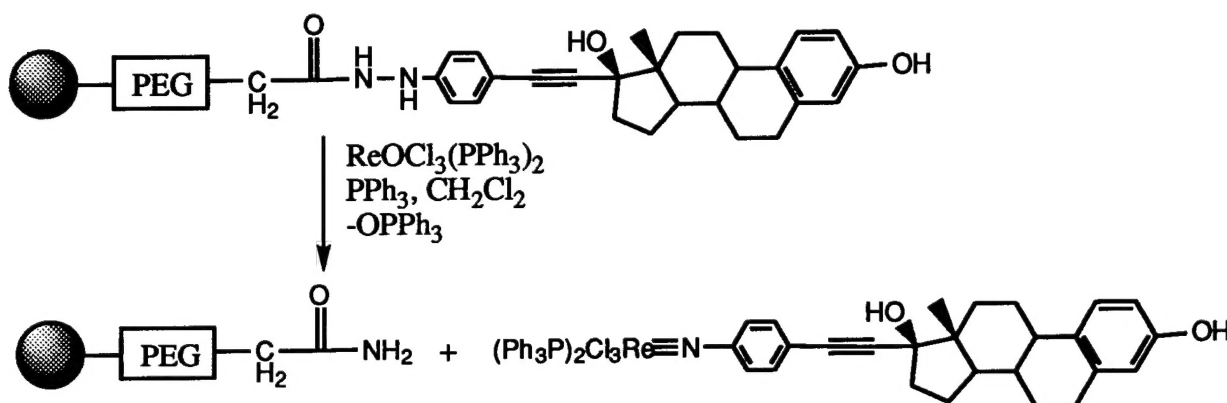
Scheme 4. Solution Phase Synthesis of Organoimido Rhenium-Estradiol Complexes

The reactions were rapid in refluxing dichloromethane solvent, and the products were obtained as green solids after precipitation with hexanes. The ethynyl- and ethenyl-linked organoimido products displayed characteristic $^{31}\text{P}\{^1\text{H}\}$ NMR signals due to the coordinated triphenylphosphine ligands at δ -20.4 and δ -20.9 respectively. Attempts to grow crystals of these complexes suitable for X-ray diffraction studies are currently in progress.

The conditions developed for the reactions in solution were then used with the solid-supported ethynyl-estradiol derivative, and produced the same product obtained from the solution reactions as

shown in Scheme 5. No other estradiol-containing fragments were detected in the crude reaction products, confirming that only the rhenium labeling step results in cleavage from the resin. This experiment provides the first example of methodology that is suitable for "instant kits" to radiolabel steroids with ^{188}Re . These results will be reported within a manuscript in preparation that will be submitted for publication in the *Journal of the American Chemical Society* within the next few months.

Scheme 5. Solid Supported Synthesis of Organoimido Rhenium-Estradiol Complexes



During this time period we also reported an X-ray structural study of a rhenium complex related to the starting compound for the organoimido syntheses $\text{ReOCl}_3(\text{PPh}_3)_2$, which is the *mer*-(triphenylphosphine)(triphenylphosphine oxide) trichlorooxo-rhenium(V) $\text{ReOCl}_3(\text{PPh}_3)(\text{OPPh}_3)$ which contains a phosphine oxide ligand.³

7. CONCLUSIONS:

The results described above demonstrate our new palladium-catalyzed synthetic routes to alkynyl- and alkenyl-linked estradiol hydrazine derivatives, and that these syntheses can be used to prepare solid-supported analogs. These compounds are obtained in excellent yields. The desired reactivity of these compounds with $\text{ReOCl}_3(\text{PPh}_3)_2$ has also been shown, leading to the first generation of target organoimidorhenium-estradiol complexes. These studies provide proof of concept for the fundamental hypothesis of this project which is that solid-supported imido precursors can be used to synthesize rhenium radiopharmaceuticals without requiring additional steps to remove unlabeled ligand.

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